Physiology | Lecture 6

# Membrane potential of excitable tissues pt.2

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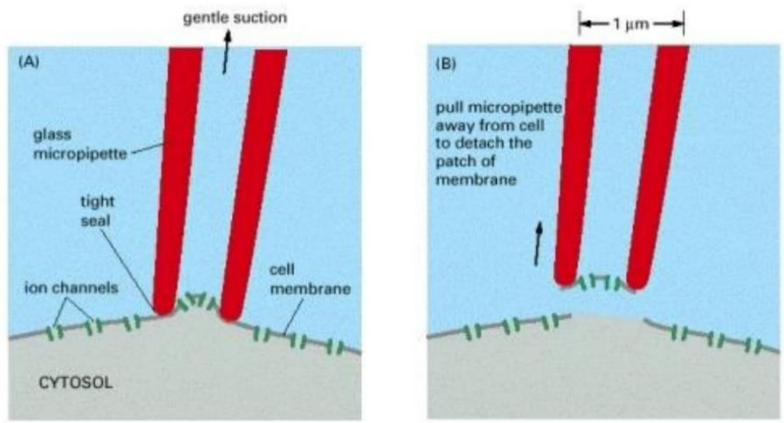
## Measuring currents at a specific membrane potential

## Patch clamp technique

**We can measure the activity of different channels at different voltages by measuring the currents of the ions that are moving.**

**Patch clamp technique: This technique helps to study the behavior of voltage gated ion channels at different membrane potentials.**

**1.The tip of the pipette that is very small, smaller than the cell, is brought close to the membrane and a gentle suction is applied to seal off a part of the membrane which contains voltage gated ion channels.**



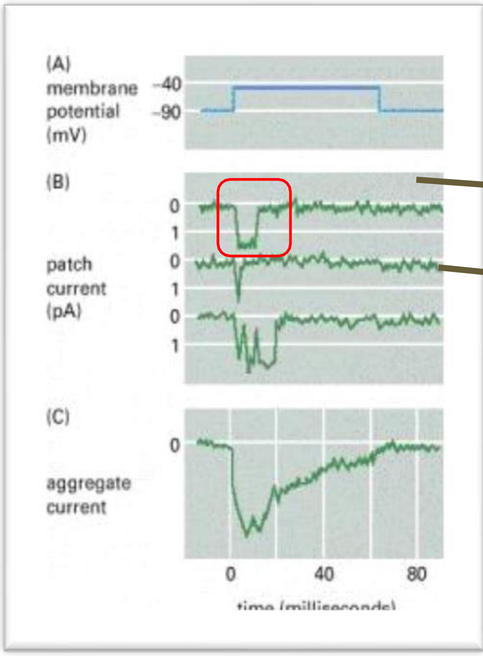

**2. A solution that is similar to the ECF ( high concentration of Na+) is placed in The pipet and then the whole tip of it is placed in a solution that is similar to the ICF ( high concentration of k+).**

**3. We can change the voltage on the membrane to observe the activity of the voltage gated channels at different membrane potentials. For example, when clamping the membrane potential at +60, there is no Na+ current, whereas there is a current at –60. This means that this channel is voltage gated and opens and closes at specific voltages.**

**Note: when clamping the potential at –95 mV which is the equilibrium potential for K+ there are no K= currents because it is at equilibrium.**

### Watch these short videos for more understanding

https://youtu.be/mVbkSD5FHOw




This picture shows the recording of currents in patch clamp.

A) Shows the voltage applied across the membrane, the potential shifts from -90mV to -40 mV indicating a depolarization.
B) Shows the activity of individual ion channels in response to voltage change, the downward deflections as the one circled in red indicates ion channel opening.
C) Shows the sum of the currents from multiple channels. The initial sharp downward deflection corresponds to a rapid opening of channels in response to depolarization

# Changes in Resting membrane potential.

We said that we can shift this potential to more or less negative.

**More negative potential:**

Called "**Hyperpolarization**"

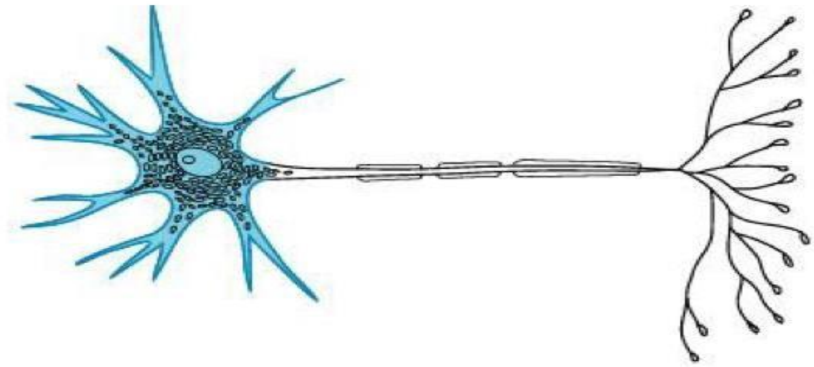
By activating more potassium channels (voltage gated) by changing the membrane potential to a specific number, it's activated by the new voltage.

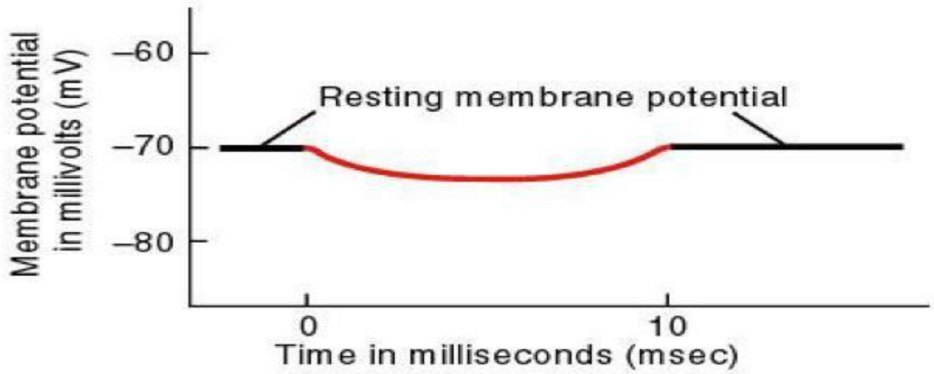
**Less negative potential:**

Called "**Depolarization**"

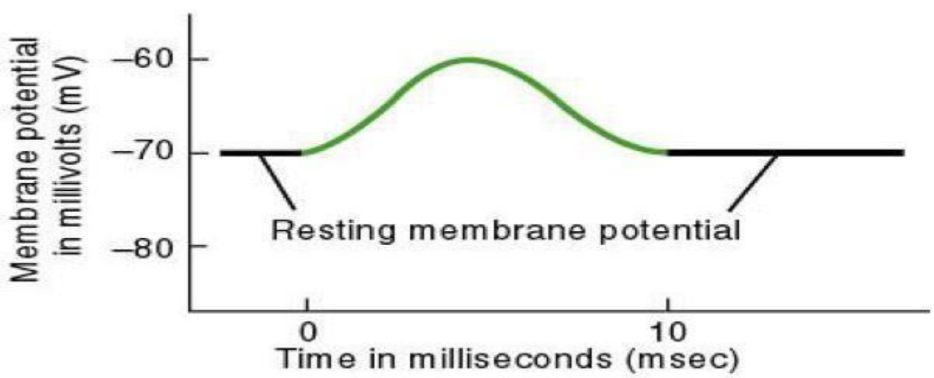
By activating more sodium channels (chemical)**.**

When activating some of these channels we will reach a point –threshold– at that point all voltage gated sodium channels are opened, so the membrane potential will be less negative and after reaching a voltage near to the equilibrium potential for sodium (+10mv to +20mv usually) these channels will be inactivated (**the conductance for Na decreased**) and this potential motivate $K^+$ channels to open.






(a) Hyperpolarizing graded potential




(b) Depolarizing graded potential

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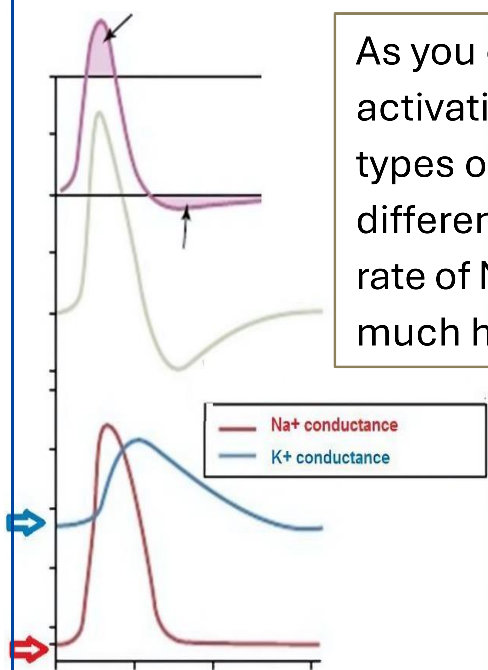
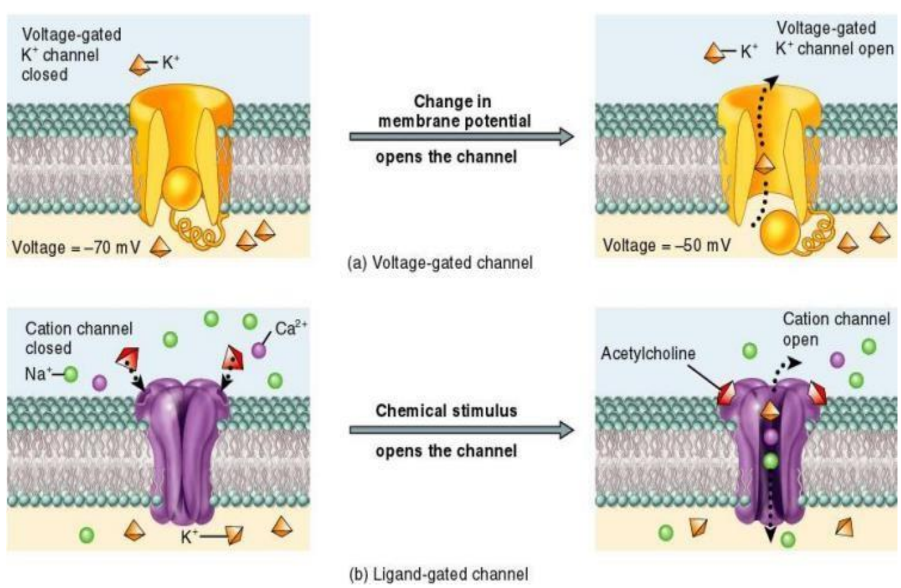



As you can see, the rate of activation for these two types of channels are different, the activation rate of $Na^+$ channels are much higher than the $K^+$

**The conductance for k+ is about 200 more than Na+**

**In other words : The resting potential is very cloooose to the Equilibrium potential for K+ .**

**At resting potential, there is very high conductance for potassium and very low conductance for sodium ions and these conductances may change according to the change in voltage**



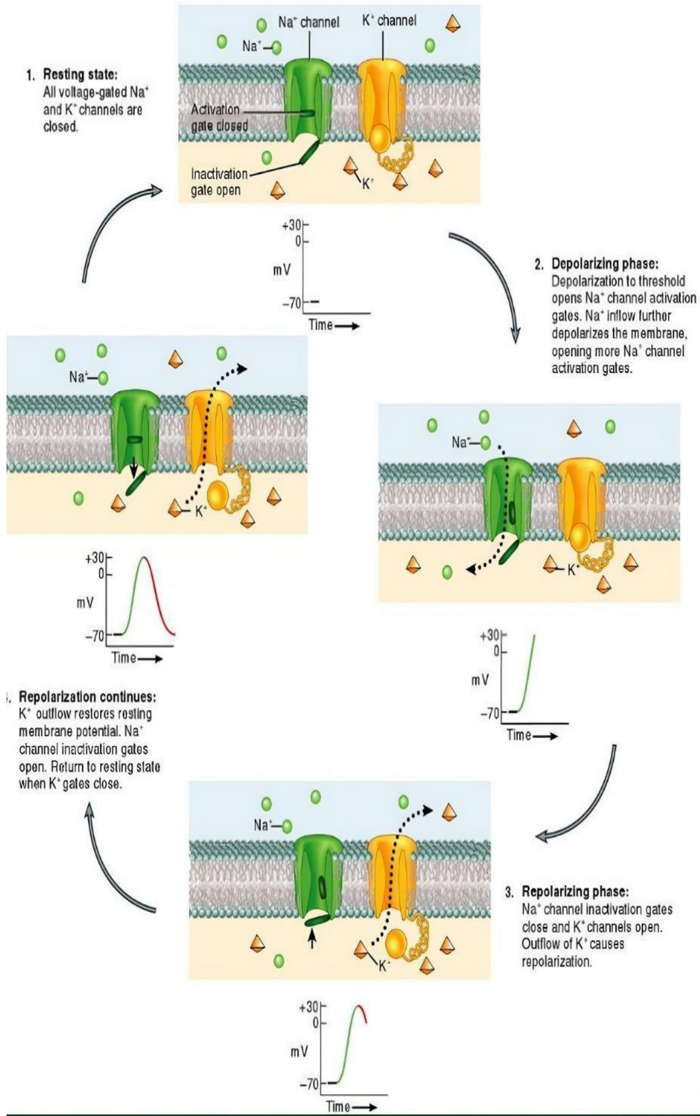

There are two types of channels:

1-**Voltage-gated channel**:

Changes its behavior according to the change in voltage across the plasma membrane.

2-**Ligand-gated channel**:

Changes its behavior if a ligand is bound to the receptor on it.




1- Resting: Both Na$^+$ and K$^+$ channels are closed.

2- Depolarizing: Na$^+$ channels are opened, Na$^+$ ions' flow toward inside increases, and this leads to more activation of Na$^+$ channels.

3- Repolarizing: Na$^+$ channels get inactivated, K$^+$ channels are opened, K$^+$ ions' flow toward outside increases, that leads the membrane to get back to the resting state, after that K$^+$ channel will be inactivated.

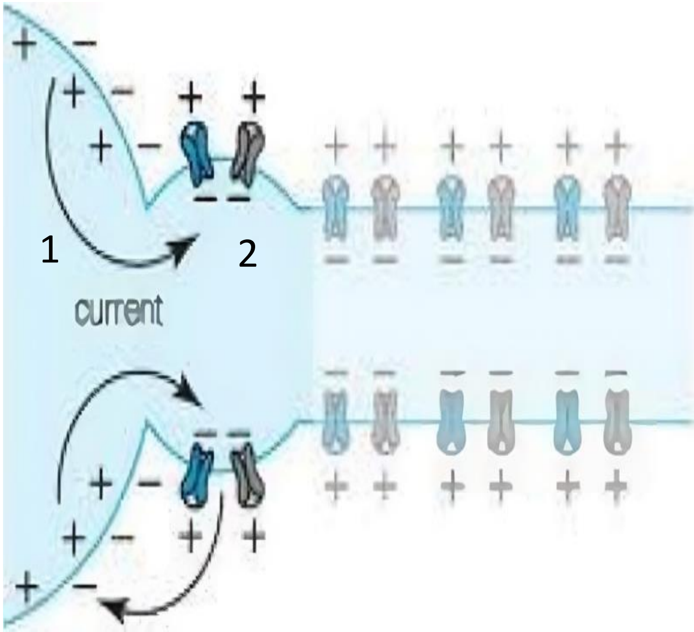
Note: Resting state is called polarized state too, so

"Repolarizing" means regenerate the polarized state, which is resting state.

But, before the action potential takes place, we must overcome the threshold, how is that happening? 1-**Ligands**. 2-**Ionic Currents**.

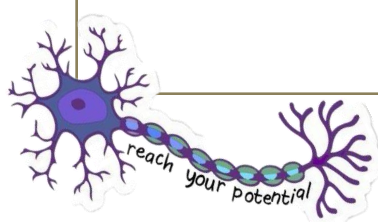
At this point, we should mention the non or all principle, that means if these Ligand or Ionic Current couldn't reach the threshold, nothing will happen because it couldn't activate the voltage-gated sodium channels, but if they could reach the threshold, the depolarization will start (by activating voltage-gated sodium channels) leading to the action potential (**<u>none or all principle</u>**).

We've already talked about Ligands, what about Ionic Currents?



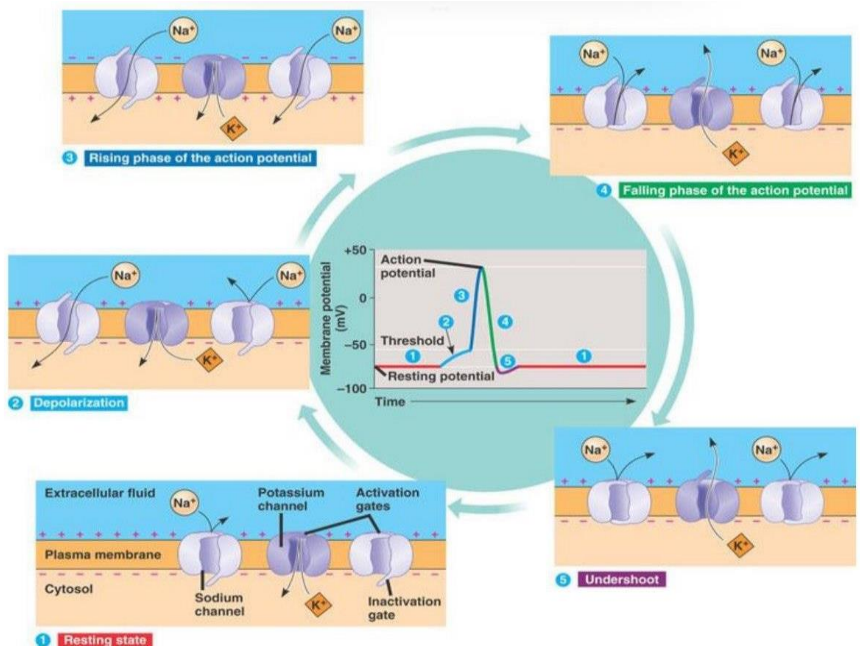

Assuming part 1 is in action potential, it becomes positive inside, and part 2 is still in the polarized state, so it's negative inside, if some positive ions (ionic current) moved from 1 toward 2, and more positive ions moved from outside toward inside part 2, it will overcome the threshold, leading to start the depolarization.

<u>In conclusion, you don't have to activate all channels over membrane manually (by Ligands), it's enough to activate one part and the action potential can move along the membrane by ionic currents.</u>



# Action potential

- As we talked in the previous sheet, sodium channels must be activated by chemical, electrical or mechanical stimuli to reach the threshold, which is called the depolarization state (in which the membrane becomes less negative inside because of the sodium ions movement toward inside).




At 1 sodium channels start to open. But

At 2 they are having the highest rate and there's a big difference between the two ☺

1) Resting state: all channels are closed ($Na^+$ and $K^+$ voltage gated channels).

2) Threshold: activation of some fast $Na^+$ chemical gated channels by stimuli and potassium gated ones at slower rate, if the stimuli were efficient enough the region will reach a specific potential "threshold" that triggers the action potential (the sodium rate is higher than the potassium).

3) Firing phase, also called Depolarization ( rising phase of the action potential): more sodium voltage gated channels will open motivated by threshold (while $K^+$ channels are still closed) causing the fast entrance of sodium ions into the cytoplasm (increasing the $Na^+$ permeability), that causes a reduction in the negative potential of the membrane ( becomes less -ve) even more because of the sodium ions positive charges causing overshoot (The reverse of the membrane potential (becomes positive inside and negative outside) where Na+ ions try to reach equilibrium. At his point, the membrane has reached maximal exchanges.

4) Repolarization (falling phase of the action potential): after the firing phase, caused by the Na+ ions, the membrane potential reaches a specific positive value ,this new potential inactivate the sodium channels and motivate the opening of k+ voltage gated channels that transport these ions from the cytoplasm to the ECM ( the cytoplasmic side is losing the positive potassium ions to the outer surface of the membrane) making the inside more negative than the outside ( increasing the membrane potential making it more -ve).

5) Undershoot (After hyperpolarization/Positive after Potential -old name): the potassium channels will close relatively slowly causing this "undershoot" hyperpolarization potential that is more negative than the resting one, this new potential inactive the potassium channels and the region will be back to its resting state after a refactoring period.

Notes:

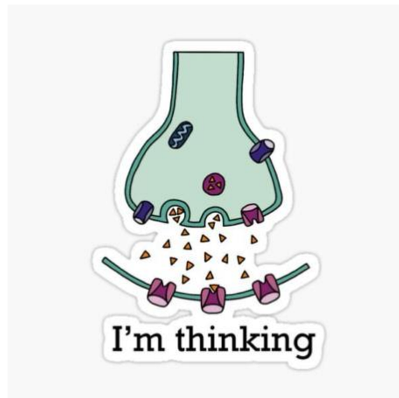
- Hyperpolarization (the general term) and after hyperpolarization are very related terms, but the doctor emphasized after hyperpolarization which occurs at the end of an Action Potential, it's a much more accurate term when talking about action potential.

- Excitation: bringing the membrane potential closer to the threshold level, triggering an action potential.
  Inhibition: pushing the membrane potential away from threshold, by making the inside more negative than the outside, increasing the refractory period.

Why the hyperpolarization has this old name?

Recording the action potential means recording the inside compared to the outside and because the first recordings of the membrane potential was according to the outside compared to inside it was named like that (reversal direction of diagram)—> you return down to reach the threshold then go further down for the depolarization then move back up for the repolarization so it's a mirror image upside down.

***Important note:*** **if the depolarized membrane didn't reach the threshold potential that triggers the action potential the membrane potential will return to the resting state.**




Note: After-hyperpolarization increases the refractory period. After hyperpolarization is due to the prolonged opening of the voltage gated K+ channels causing an efflux of extra K+ ions outside the cell, making the interior more negative and moving the membrane potential further away from the threshold, making it more distant from the threshold, which means it will take longer time to reach the threshold, thus making the refractory period longer.

Threshold: The point that must be attained by opening the ligand gated Na+ channels to undergo depolarization due to the amplified opening of voltage gated Na+ channels by a positive feedback mechanism.

Refractory point: The period of time that it takes an excitatory cell to recover from a former action potential and to be able to fire another action potential again.

It may be relative (when sodium ion voltage-gated channels are closed and not capable to open. It can be stimulated only by a strong nerval impulse) or absolute (when sodium ion voltage-gated channels are all opened. It can't be stimulated at all.).

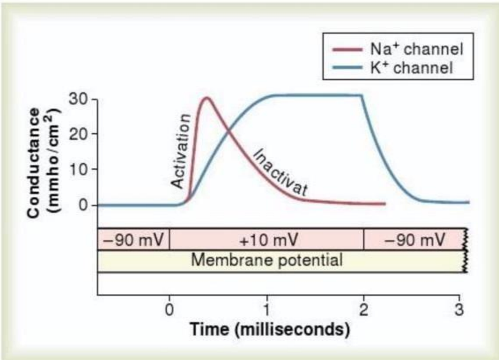


Figure 5–9

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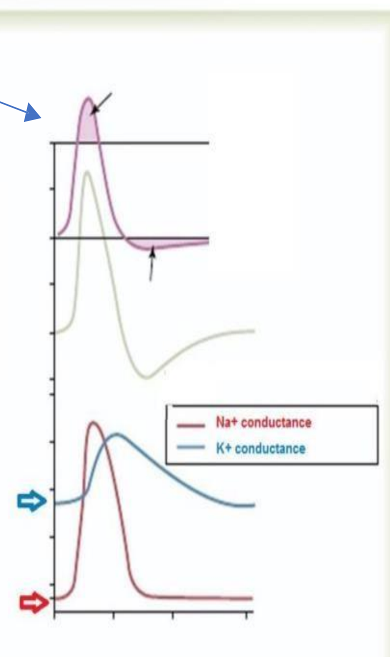
Overshot: is the peak of the action potential where's the membrane potential is positive it may be the period in black or all the red curve.

This drawing refers to the ratio of the conductance for both ions.




- Na+ and K+ conductance at resting potentials

You are rating the conductances with what's happening in the action potential at the start we have a higher conductance for potassium but opening the sodium channels changes the case.

At resting potential, the conductance of K+ is more than Na+

If stimuli affect the cell in other periods, it will not respond cause the channels are still open during action potential.

***Please check on the professor's handout, Very Important ..***

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Department of Physiology & Biochemistry

**Introduction Med**, 2023/2024

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# Membrane physiology and the basis of excitability

Ref: Guyton, 14th ed. 63-76, Jordan and 13th ed. pp: 61-71. 12th ed. pp: **57-69**,

## MEMBRANE POTENTIALS AND ACTION POTENTIALS:

### MEMBRANE POTENTIAL:

If we assume that a cellular membrane is permeable **only** to K+, which is found in a very high concentration inside the cell. K+ will diffuse to the extracellular fluid because of the concentration gradient. The diffusion of K+ will result in a movement of positive charges outside the cell and leaving behind negative charges inside the cell. This will create an electrical potential difference across the membrane (positive outside and negative inside). Creation of this potential difference will oppose diffusion of K+ to the outside at a certain concentration difference. When you reach a point at which diffusion of K+ is completely opposed by the potential difference created across the membrane and the net diffusion for K+ is zero even though you still have a concentration gradient, you have reached the equilibrium potential for K+ ($E_K$). The equilibrium potential for any univalent ion at normal temperature can be calculated by Nernest equation:

E (mV) = - 61.log (Ci/Co)

E = equilibrium potential for a univalent ion
Ci = concentration inside the cell.
Co = concentration outside the cell.

When more ions are involved in creating the potential, we can calculate the potential according to Goldman-Hodgkin-Katz equation.

$$E_m = \frac{RT}{F} \ln \left( \frac{P_{Na^+}[Na^+]_o + P_{K^+}[K^+]_o + P_{Cl^-}[Cl^-]_i}{P_{Na^+}[Na^+]_i + P_{K^+}[K^+]_i + P_{Cl^-}[Cl^-]_o} \right)$$

P = permeability of the membrane to that ion.

In this equation, Goldman and his colleagues considered that these ions are mostly involved in the development of membrane potential.

According to this equation, the permeability of the membrane to an ion is very important in determining the membrane potential. If the membrane is permeable only to K+ and not permeable to Cl- and Na+, the membrane potential will be equal to $E_{K+}$.

## Resting membrane potential:

In excitable cells the membrane potential is not constant. When the cell is stimulated, the membrane potential is changing. These changes in membrane potential are due to changes in permeability of plasma membrane to different ions. For example, when a neuron is stimulated, this will result in increased permeability to Na+. This will bring the membrane potential closely to $E_{Na}$. The recorded membrane potential for a cell under resting conditions when no stimulus is involved is known as **resting membrane potential**. For neurons, the recorded resting membrane potential is about (– 90 mV). This represents a potential difference between the inside to the outside when the neuron is not active.

### Origin of resting membrane potential:

Contribution of K+ diffusion:

As mentioned earlier, if the membrane is permeable only for K+ the calculated $E_{K+}$ is about (-94mV).

$Co_{K+}$ = 4meq/l , $Ci_{K+}$ = 140meq/l

$E_{K+} = -61. \log 140/4 = -94mV$

Which is not far from the recorded membrane potential but not exactly.

The contribution of Na+ diffusion:

Membrane is also permeable to Na+. The permeability of the plasma membrane for Na+ is much less than that of K+. If the membrane is permeable only to Na+, the calculated $E_{Na+}$ = + 61mV.

……….. ($Co_{Na+}$ = 142meq/l , $Ci_{Na+}$ = 14meq/l).

Because of the permeability of the membrane for the two ions, the E would be between (-94mV and +61mV). The calculated E for the two ions is –86mV, which is not far from the $E_{K+}$ because of the higher permeability of membrane for K+ than for Na+ (100 times more).



So the Na+ contribution in resting potential is by bringing the membrane potential to a lower value than the calculated $E_{K+}$.

Contribution of Na+ - K+ pump:

As mentioned earlier, this pump is electrogenic. It moves more positive charges outside the cell (3 for 2). This will induce loss of positive charges from the cell and bring the membrane potential to a higher negativity (about –4mV additional negativity).

Therefore all these factors, during **rest**, will give a net membrane potential of –90mV (called **Resting Membrane Potential**).

## ACTION POTENTIAL:

As we have seen, the plasma membrane is **polarized** (has ability to separate opposite charges) during resting state. When the membrane potential decreases (becomes less negative), the membrane is in **depolarization** stage. While the change in membrane potential in opposite direction (becomes more negative than resting potential) is known as **hyperpolarization**.

When a cell is depolarizing, it reaches a maximum according to stimulus, then the membrane potential returns to its resting state. The phase of returning from depolarized state to resting state is known as **repolarization**. These changes in membrane potential can be recorded by placing one electrode inside the cell and the other outside the cell. By recording of whole action potential in this way, we will obtain a **monophasic action potential**.

Let us consider the changes in membrane potential of an excitable cell to understand the events that appear during changes of membrane potential. To induce a change, a stimulus must be applied to change activity of channels at the membrane. Any increase in permeability of membrane to Na+ will result in diffusion of (+) charges inward. This event will decrease the membrane potential (becomes less negative). And conversely any increase in K+ diffusion (movement outward) will result in an increase in membrane potential (becomes more negative). The diffusion of these ions depends on the activity of Na+ and K+ channels that are found on the membrane. Activation of Na+ channels will induce depolarization, while activation of K+ channels will increase the potential difference across membrane

**Action potential and the role of Na+ channels:**

On the membrane, most Na+ channels during resting state are inactive (closed). According to channel type, these channels can be activated by a chemical stimulus (in case of chemical gated channels), electrical stimulus (in case of voltage gated channels), or mechanical stimulus. In the case of chemical gated channels, binding of ligand to its receptor will induce activation of chemical gated Na+ channels. Once activated, the membrane potential will decrease (becomes less negative). Which means that the membrane depolarizes. The voltage changes in the membrane will cause the other type of channels (Na+ voltage gated channels) to be activated. Activation of these channels will cause more changes in membrane potential (more depolarization). More and more depolarization will occur in the membrane by a positive feed back mechanism. If we reach a point at which most voltage gated Na+ channels are activated, this will cause a sudden increase in Na+ permeability. This increase in Na+ permeability will even reverse the membrane potential (becomes positive inside and negative outside) (this is known as the **overshot** in the action potential), because Na+ is trying to approach its equilibrium potential ($E_{Na}$). At this point, the membrane has reached maximal changes in membrane potential (a peak of an action potential).

As we have seen, during depolarization there is a point at which a sudden increase in Na+ influx which induces rapid and maximal change in membrane potential. This point is known as the **threshold** of an action potential. The rapid change in membrane potential during the raising phase of an action potential is known as **firing stage**. When a stimulus causes a depolarization that brings the membrane potential to the threshold, the membrane will respond by the firing stage of an action potential. If depolarization in the membrane has not reached threshold, the membrane will not enter firing stage, and instead, the potential returns to its resting level. Therefore, the response in the membrane will be either by an action potential when threshold is achieved or no appearance of an action potential when the membrane potential has not reached threshold. For that reason, induction of an action potential in excitable cells follows the **NONE OR ALL PRINCIPLE**.

The voltage changes in membrane potential not only activate voltage dependent Na+ channels, but also inactivate these channels at certain potential difference. This inactivation appears because channels have changed their state from opened channels to closed channels due to voltage changes. The closing event of Na+ channels does not make these channels as the only responsible for bringing membrane potential to its resting level. But also, activation of voltage dependent K+ channels is the main player in returning the membrane potential to its resting level.

**Action potential and K+ channels:**

Although there is some leakage of K+ during resting state, which maintains the resting membrane potential close to $E_{K+,}$ depolarization causes activation of voltage gated K+ channels. The activation of these channels is much slower than activation of Na+ channels. This results in a delay in the maximal activation of K+ channels.

The delayed activation of K+ channels combined with inactivation of Na+ channels will result in a rapid returning of the membrane potential to its resting level, causing the **falling phase** in the action potential. The membrane potential may go for a while to more negative potential than during resting potential, which is known as **positive afterpotential** (**after hyperpolarization**). Followed by full recovery in the membrane potential (returns completely to its resting level). The positive after potential is probably due to an excess in K+ efflux, which causes more deficit of positive ions inside the cell.

**Action potential and Ca++:**

As discussed before, the raising phase of an action potential results by fast activation of Na+ channels. These are called *fast channels*. In some excitable cells, like cardiac muscle and uterine muscle, cells are equipped with another type of channels known as *slow* Na+ – Ca++ *channels*. These channels are activated at slower rate than Na+ channels. The slow and prolonged opening of slow channels will cause mainly Ca++ to enter the cell and prevents the rapid fall induced by activation of K+ channels, and the membrane potential is maintained for a while then the potential falls to its resting level. This is known as a **plateau** in action potential. The presence of plateau in this type of cell is important in prolonging the time of an action potential, giving more time for the cell to be able to respond to another stimulus, because the cell remains longer time in **refractory period**.

**Refractory periods of an action potential:**

During an action potential, the cell is not able to respond to another stimulus. From the firing stage to the end of the first third of falling phase the cell will not respond at all even by a stronger stimulus. In this stage the cell is said to be in **absolute refractory period**. From the beginning of the second phase until the resting membrane potential is achieved, the cell cannot respond to the usual stimulus, but a stronger stimulus can change the membrane potential. In this period, the cell is in **relative refractory period**.

The periods depend on the activity of Na+ channels. These channels pass three states during action potential. During resting potential, Na+ channels are **closed but capable for opening** when stimulated. During the raising phase (firing), almost all Na+ channels are **opened**. And any other stimulus (even stronger one) will not cause activation of more Na+ channels. During this period, the membrane is in absolute refractory period.

In the third state, when voltage dependent Na+ channels become closed after the membrane potential has reached positive values. At this state, Na+ channels are not capable for opening. During all the falling phase of an action potential, these channels remain **closed and not capable for opening**. They can pass to the first state (closed and capable for opening) when the membrane potential returns to its normal level or to a more negative potential than resting potential. During this period, the membrane is in relative refractory period. This means that a stronger (suprathreshold) stimulus may activate the closed channels that are not capable for opening by normal stimulation. In addition to the role of voltage gated Na+ channels in establishing the relative refractory period, the presence of widely opened K+ channels during falling phase, which cause excess flow of positive charges to the outside, may also play a role by opposing stimulating signals.

**Na+ -K+ pump and action potential:**

This pump has **no** role in the electrical activity that are taking place during action potential. But it plays an important role in restoring ionic composition that has been altered during action potential. This role is important in maintaining the ionic composition of the intra- and the extra-cellular fluids.

# Additional Resources

رسالة من الفريق العلمي:


For any feedback, scan or click the code.

| Versions | Slide # | Before | After |
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| V0 → V1 | | | |
| V1 → V2 | | | |